Supplementary Data

Characterization of a Factor H mutation that perturbs alternative pathway cofactor and decay acceleration activity in a family with MPGN

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Clinical History

This family was first described by Power *et al.* in 1990[1] (Figure 1). Patient 1:1 was healthy with no APL. Urinalysis, 24 hr urinary protein estimation and serum biochemistry were normal. Patient 1:2 presented with nephrotic range proteinuria and hypertension during the 2nd trimester of pregnancy and underwent a termination. She had 5 previous pregnancies all complicated by proteinuria and hypertension with 4 successfully completed to term. Renal biopsy demonstrated MPGN (intense IgM and less intense IgG staining)[1]. She had APL.

Patient 2:1 presented aged 17 with proteinuria, microscopic haematuria, normal renal function, low C3 levels and APL. A renal biopsy demonstrated MPGN. By the age of 23 he had developed nephrotic syndrome and his renal function had deteriorated with a creatinine of 155 µmol/l. By age 25 he was commenced on haemodialysis. He received a cadaveric renal transplant (prednisolone, azathioprine, cyclosporine) which failed after 7 years.

Patient 2:2. had a spinal injury aged 39 which resulted in a complete T10 paraplegia. He also had diabetes mellitus. By the age of 40 he had renal impairment (creatinine 140 µmol/l) and proteinuria and by age 41 he had commenced long term renal replacement therapy. He did not have a renal biopsy. He died aged 43 of sepsis secondary to an infected pressure sore. He had not developed APL.

Patient 2:3 remains healthy aged 45 with no proteinuria, haematuria and normal renal function.

Patient 2:4 was referred aged 8 with APL. Urinalysis was unremarkable and the renal function was normal. Aged 25 he presented with arthritis of both knees. Aged 33 he presented with multi-system symptoms and was found to have low C4 levels, anti-nuclear antibodies and double stranded DNA antibodies. His renal function remains normal with a creatinine of 61 µmol/l and normal urinalysis.

Supplementary Methods

Factor H autoantibody assay

This assay was performed as previously described[2]. Flexible 96-well plates were coated with 5µg/ml of purified fH (Merck) in pH 7.6 coating buffer (AbDserotec) (overnight 4°C). Plates were washed thrice with PBS/Tween 0.01% (PBS-T) and blocked with ultrablock (20°C)(AbDserotec). A duplicate plate was then set up with block solution as a background binding control. After blocking, a 1/50 dilution of sera in PBS-T was loaded (50µl) in triplicate for 1-2 hrs. Plates were washed thrice and then blocked as above. Goat anti-human IgG horse radish peroxidase (HRP, Stratech Scientific) at 1/20,000 was then added and incubated for 1 hr at room temperature. Plates were then washed twice with PBS-T. Tetramethyl benzidine (TMB) standard kinetic solution (AbDserotec) was then added for 7 min and stopped using 10% sulphuric acid. Absorbance at 450 nm (OD450) was established using a SpectraMax 190 plate reader (MDS Analytical Technologies Limited). Triplicate data were analysed and mean ultrablock only readings subtracted from mean fH readings to control for non-specific/false positive readings. A standard curve was generated from a known positive sample (Dr M. Dragon-Durey) and the OD450 value for the 1/25 dilution assigned 4,000 relative units. Linear-regression curve fit analysis was performed and RU values calculated using GraphPad Prism (version 3).

Whole exome sequencing

For investigation of APL genetics, whole exome sequencing was performed. 1µg dsDNA was used for TruSeq genomic DNA library prep (Illumina). First the DNA was fragmented (Covaris), giving a target size of 200-300 bp. The ends were repaired (Klenow reaction), 3' of fragments are adenylated and ligated to adapters. A final PCR amplification produces double stranded fragments with 5' and 3' adapters. 500ng library was used in TruSeq exome enrichment kit (Illumina). Libraries are denatured and added to the flow cell, which is sequenced on a HiSeq 2000, TruSEq v3. Sequence was aligned to the human reference genome (UCSC hg19) with the use of BWA[3], then reformatted with the use of SAMtools[4]. 87% of exon target sequence was covered by > 10 reads with an average coverage of 90 reads. Single base variants were identified with Varscan[5], and Indels were identified with Dindel[6]. Lists of on-target variants were filtered against dbSNP131 and the exome sequences

of 10 unrelated and unaffected persons. Putative disease-causing mutations were identified with Mutation Taster[7].

Confirmation of R83S mutation from formalin-fixed, paraffin-embedded tissue sections

Although no DNA was available from 1:2, a formalin-fixed paraffin-embedded tissue block was available from this individual. We attempted to extract DNA from the tissue block using a 5 minute incubation at 75°C prior to an overnight incubation at 55°C with proteinase K incubation. This was followed by automated purification on the QIAsymphony SP robot with DSP DNA Mini kit reagents (Qiagen). On all three occasions no quantifiable DNA was obtained and PCR failed to yield a product for the exon of interest.

Subsequently the REPLI-g FFPE kit QIAamp DNA FFPE Tissue Kit was used to whole genome amplify DNA from the formalin-fixed paraffin-embedded tissue. New primers were designed to produce an amplicon size optimized for whole genome amplified DNA. (GTA GCG CGA CGG CCA GTC TCC TAC ATA AAA TAT ATT C; CAG GGC GCA GCG ATG ACC CAA AAG GAG TAT CTC CAG GAT G). This was purified and Sanger sequenced .

Supplementary Data

Factor H autoantibody

In ELISAs to measure fH autoantibodies, individuals 2:1 and 2:4 gave an average RU value above 100 RU, the cut-off used to identify the presence of fH autoantibody, after 4 experiments (Figure S1a). A western blot was then used to confirm positive autoantibody signal. Only individual 2:4, the individual with the highest titre in ELISA, was clearly positive using this technique (Figure S1b). Autoantibody epitope mapping demonstrated that in individual 2:4, a polyclonal response was seen with binding predominately to complement control protein modules (CCP) 1-4, CCP6-8 and CCP15-18 (Figure S1c). This analysis also confirmed low, polyclonal reactivity of individual 2:1 across many fragments of fH. These data are similar to another recently reported individual with MPGN and fH autoantibodies.

Thus, 2:4 has high titre fH autoantibodies, at least equivalent to a recently characterised patient with MPGN 1[8] but serum fH levels remain in the normal range (Table S1). The autoimmune disease noted in the clinical history of 2:4 suggests, in this individual, that the presence of both C3Nef and fH autoantibodies stems from a predisposition to autoimmunity. Indeed, a recent study has indicated that 11.5% of rheumatoid arthritis (RA) (n=314) and 6.7% of systemic lupus erythematosus (SLE) patients (n = 60) have detectable autoantibodies to fH (as well as 4% of normal individuals; n = 354) [9]. Their data also indicated that unlike the fH autoantibodies found in ~10% of aHUS patients (where autoantibodies predominately target the C terminus), autoantibodies in RA and SLE were polyclonal with respect to epitope binding. Bringing this together, our data fit with individual 2:4 having fH autoantibodies in association with SLE and thus, the presence of fH autoantibodies is unlikely to be directly linked with the development of kidney disease in this family.

Supplementary Table S1

	1:1	1:2	2:1	2:2	2:3	2:4
CFH genetic analysis	N/P	R83S	R83S	R83S	WT	WT
CFH Haplotype	N/P	N/P	H3/H5	*CFH* H3/H5	N/P	*CFH* H3/H3
MCP gaggt haplotype	N/P	N/P	1 copy	1 copy	N/P	2 copies
MCPaaggt haplotype	N/P	N/P	0 copies	0 copies	N/P	0 copies
C3 R102G	N/P	R/V	R/V	V/V	R/R	R/V
C3 P314L	N/P	R/V	R/V	V/V	R/R	R/V
C3 (0.68-1.38 g/l)	0.87*	0.10*	0.73 φ	0.61 φ	1.87 φ	0.24 φ
C4 (0.18-0.60 g/l)	0.88*	1.01*	0.19 φ	0.18 φ	N/A	0.13 φ
CFH (0.35-0.59 g/l)	N/A	N/A	0.76	0.71	N/A	0.52
CFI (38-58 mg/L)	N/A	N/A	58	62	N/A	59
CD46	N/A	N/A	Normal	Normal	N/A	Normal
C3Nef	-*	+ *	+ *	-*	N/A	+ *
αCFH Ab	N/A	N/A	- φ	- φ	- φ	+ φ

Supplementary Table S1. Complement Assay results. N/P- genetic analysis not performed; WT *CFH* genetic screening normal; R83S- mutation present in heterozygosity; R- Reference sequence; v – Variant sequence. N/A- not available. § Although a positive result was seen on ELISA, confirmatory western blot was negative. No patient carried the MCP_{aaggt} haplotype associated with C3GN and MPGN1[10]. Patients 2:1 and 2:2 both carried one copy of the MCP_{gaggt} previously associated with aHUS[11-13] and 2:4 had 2 copies of this MCP_{gaggt} haplotype. * Measured 1990. φ Measured 2009.

Supplementary Table 2

Gene Name	Symbol	Reference
Partial lipodystrophies associated genes		
Lamin A/C	*LMNA*	[14]
Peroxisome proliferator-activated receptors γ	*PPARG*	[15]
Zinc metalloproteinase STE24	*ZMPSTE24*	[16]
Perilipin	*PLIN1*	[17]
Nuclear lamina protein lamin B2	*LMNB2*	[18]
Protease subunit, beta type 8	*PSMB8*	[19]
Total lipodystrophy associated genes		
Seipin	*BSCL2*	[20]
Lysophosphatidic acid acyltransferase	*AGPAT2*	[21]
Caveolin1	*CAV1*	[22]
Polymerase I and transcript release factor	*PTRF*	[23]

Supplementary Table S2 Lipodystrophy associated genes. The genes listed above have been associated with partial and complete lipodystrophies. Whole exome sequencing was performed to screen these genes however no genetic variants were identified which segregated with lipodystrophy in the family.

Figure S1. Complement Factor H Autoantibodies. (a) The positive cut-off (O.D. 450= 0.2) [Horizontal line] is derived from the 97.5 percentile of the autoantibody titre in the control population[8]. (b) Purified fH was run on 10% SDS-PAGE, transferred to nitrocellulose and strips incubated with patient and control sera as indicated above panel. Intervening irrelevant strips of the screening blot have been excised as indicated by T bar. Representative of 2 such blots. Only individual 2:4, the individual with the highest fH autoantibody titre was positive. (c) Epitope mapping was undertaken using short fragments of fH [CCPs1-4[24], 6-8[25], 8-15[26], 15-18[27], 18-20[28] and fHR1 CCP4-5[29])] using the same ELISA protocol as for purified fH. Molar equivalents were coated onto ELISA plates, and a BSA subtraction performed.

Supplementary References

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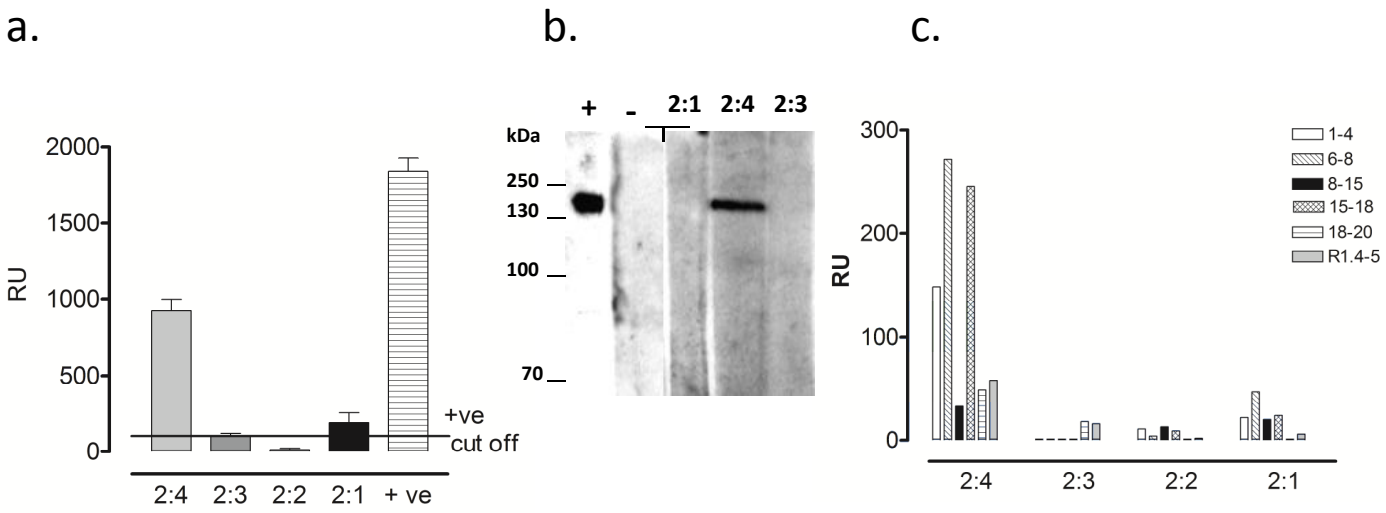
Figure S1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